



REF 10302

PR3



Instruction Manual

Table of Contents

1	Intended Use	1
2	Clinical Application and Principle of the Assay	1
3	Kit Contents	2
4	Storage and Shelf Life	2
5	Precautions of Use.....	3
6	Sample Collection, Handling and Storage.....	4
7	Assay Procedure	4
8	Quantitative Interpretation.....	7
9	Technical Data.....	8
10	Performance Data.....	8
11	Disposal.....	9
12	Literature	9



AIDA GmbH
Dr.-Karl-Aschoff-Straße 9
55543 Bad Kreuznach
Germany
Phone: +49 671 92065090
Fax: +49 671 92065091
Website: www.aida-diagnostics.com
Mail: info@aida-diagnostics.com

	Product Ref.	10302
	Product Desc.	PR3
	Manual Rev. No.	006: 2024-02-13

1 Intended Use

PR3 is a solid phase enzyme immunoassay employing highly purified native human proteinase 3 (PR3) from human neutrophil granulocytes for the quantitative detection of antibodies against proteinase 3 in human serum. Anti-PR3 antibodies recognize specifically conformational epitopes only accessible on native PR3.

The assay is an aid in the differential diagnosis of autoimmune vasculitis.

2 Clinical Application and Principle of the Assay

Antibodies against proteinase 3 (PR3) belong to the group of anti-neutrophil cytoplasmic antibodies (ANCA) which are directed against cytoplasmic components of neutrophilic granulocytes and monocytes. Indirect immunofluorescence test on ethanol-fixed neutrophils has been the established method for the detection of ANCAs. It became apparent that some ANCAs create a cytoplasmic fluorescence pattern (thus called cANCA) while others create a perinuclear pattern (the pANCA). As both patterns may cover multiple antigens, immunofluorescence is not suitable for a satisfying differential diagnosis of vasculitis; thus each IFT should be verified with specific ELISA tests.

PR3 is a serine protease from the azurophile granules (lysosomes) of neutrophils with a molecular weight of 29 kDa. The cationic protein has a proteolytic activity to elastin, hemoglobin and collagen VII. In addition, PR3 promotes the activation of platelets by cathepsin G and inactivates the C1-inhibitor.

ANCA are important markers for the differential diagnosis of autoimmune vasculitis. Autoantibodies to PR3 are a specific serological marker for Wegener's granulomatosis (WG). Although the etiology and pathogenesis is still obscure, autoantibodies to PR3 are thought to play an active role in the pathogenesis of WG. Anti-PR3 titers are strongly associated with disease activity and inhibit the proteolytic activity of PR3.

Principle of the test

Serum samples diluted 1:101 are incubated in the microplates coated with the specific antigen. Patient's antibodies, if present in the specimen, bind to the antigen. The unbound fraction is washed off in the following step. Afterwards anti-human immunoglobulins conjugated to horseradish peroxidase (conjugate) are incubated and react with the antigen-antibody complex of the samples in the microplates. Unbound conjugate is washed off in the following step. Addition of TMB-substrate generates an enzymatic colorimetric (blue) reaction, which is stopped by diluted acid (color changes to yellow). The intensity of color formation from the chromogen is a function of the amount of conjugate bound to the antigen-antibody complex and this is proportional to the initial concentration of the respective antibodies in the patient sample.

	Product Ref.	10302
	Product Desc.	PR3
	Manual Rev. No.	006: 2024-02-13

3 Kit Contents

TO BE RECONSTITUTED				
Item	Quantity	Cap color	Solution color	Description / Contents
Sample Buffer (5x)	1 x 20ml	White	Yellow	5 x concentrated Tris, sodium chloride (NaCl), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Wash Buffer (50x)	1 x 20ml	White	Green	50 x concentrated Tris, NaCl, Tween 20, sodium azide < 0.1% (preservative)
READY TO USE				
Item	Quantity	Cap color	Solution color	Description / Contents
Negative Control	1 x 1.5ml	Green	Colorless	Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Positive Control	1 x 1.5ml	Red	Yellow	Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Calibrators	6 x 1.5ml	White	Yellow *	Concentration of each calibrator: 0, 3, 10, 30, 100, 300 U/ml. Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Conjugate, IgG	1 x 15ml	Blue	Blue	Containing: Anti-human immunoglobulins conjugated to horseradish peroxidase, bovine serum albumin (BSA)
TMB Substrate	1 x 15ml	Black	Colorless	Stabilized tetramethylbenzidine and hydrogen peroxide (TMB/H ₂ O ₂)
Stop Solution	1 x 15ml	White	Colorless	1M Hydrochloric Acid
Microtiter plate	12 x 8 well strips	N/A	N/A	With breakaway microwells. Refer to paragraph 1 for coating.
* Color increasing with concentration				
MATERIALS REQUIRED, BUT NOT PROVIDED				
Microtiter plate reader 450 nm reading filter and recommended 620 nm reference filter (600-690 nm). Glass ware (cylinder 100-1000ml), test tubes for dilutions. Vortex mixer, precision pipettes (10, 100, 200, 500, 1000 µl) or adjustable multipipette (100-1000µl). Microplate washing device (300 µl repeating or multichannel pipette or automated system), adsorbent paper. Our tests are designed to be used with purified water according to the definition of the United States Pharmacopeia (USP 26 - NF 21) and the European Pharmacopeia (Eur.Ph. 4th ed.).				

4 Storage and Shelf Life

Store all reagents and the microplate at 2-8°C/35.6-46.4°F, in their original containers. Once prepared, reconstituted solutions are stable at 2-8°C/35.6-46.4°F for at least 1 month. Reagents and the microplate shall be used within the expiry date indicated on each component, only. Avoid intense exposure of TMB solution to light. Store microplates in designated foil, including the desiccant, and seal tightly.

	Product Ref.	10302
	Product Desc.	PR3
	Manual Rev. No.	006: 2024-02-13

5 Precautions of Use

5.1 Health hazard data

THIS PRODUCT IS FOR IN VITRO DIAGNOSTIC USE ONLY. Thus, only staff trained and specially advised in methods of in vitro diagnostics may perform the kit. Although this product is not considered particularly toxic or dangerous in conditions of the intended use, refer to the following for maximum safety:

Recommendations and precautions

This kit contains potentially hazardous components. Though kit reagents are not classified being irritant to eyes and skin we recommend to avoid contact with eyes and skin and wear disposable gloves.

WARNING ! Calibrators, Controls and Buffers contain sodium azide (NaN₃) as a preservative. NaN₃ may be toxic if ingested or adsorbed by skin or eyes. NaN₃ may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. Please refer to decontamination procedures as outlined by CDC or other local/national guidelines.

Do not smoke, eat or drink when manipulating the kit. Do not pipette by mouth.

All human source material used for some reagents of this kit (controls, standards e.g.) has been tested by approved methods and found negative for HbsAg, Hepatitis C and HIV 1. However, no test can guarantee the absence of viral agents in such material completely. Thus handle kit controls, standards and patient samples as if capable of transmitting infectious diseases and according to national requirements.

The kit contains material of animal origin as stated in the table of contents, handle according to national requirements.

5.2 General directions for use

In case that the product information, including the labeling, is defective or incorrect please contact the manufacturer or the supplier of the test kit.

Do not mix or substitute Controls, Calibrators, Conjugates or microplates from different lot numbers. This may lead to variations in the results.

Allow all components to reach room temperature (20-32°C/68-89.6°F) before use, mix well and follow the recommended incubation scheme for an optimum performance of the test.

Incubation: We recommend test performance at 30°C/86°F for automated systems.

Never expose components to higher temperature than 37°C/ 98.6°F.

Always pipette substrate solution with brand new tips only. Protect this reagent from light. Never pipette conjugate with tips used with other reagents prior.

A definite clinical diagnosis should not be based on the results of the performed test only, but should be made by the physician after all clinical and laboratory findings have been evaluated. The diagnosis is to be verified using different diagnostic methods.

 autoimmune diagnostic assays	Product Ref.	10302
	Product Desc.	PR3
	Manual Rev. No.	006: 2024-02-13

6 Sample Collection, Handling and Storage

Use preferentially freshly collected serum samples. Blood withdrawal must follow national requirements. Do not use icteric, lipemic, hemolysed or bacterially contaminated samples. Sera with particles should be cleared by low speed centrifugation (<1000 x g). Blood samples should be collected in clean, dry and empty tubes.

After separation, the serum samples should be used during the first 8h, respectively stored tightly closed at 2-8°C/35.6-46.4°F up to 48h, or frozen at -20°C/-4°F for longer periods. (Thomas: Labor und Diagnose; CLSI Guideline GP44-A4)

7 Assay Procedure

7.1 Preparations prior to starting

Dilute concentrated reagents:

Dilute the concentrated sample buffer 1:5 with distilled water (e.g. 20 ml plus 80 ml).

Dilute the concentrated wash buffer 1:50 with distilled water (e.g. 20 ml plus 980 ml).

To avoid mistakes we suggest to mark the cap of the different calibrators.

Samples:

Dilute serum samples 1:101 with sample buffer (1x)

e.g. 1000 µl sample buffer (1x) + 10 µl serum. Mix well !

Washing:

Prepare 20 ml of diluted wash buffer (1x) per 8 wells or 200 ml for 96 wells

e.g. 4 ml concentrate plus 196 ml distilled water.

Automated washing:

Consider excess volumes required for setting up the instrument and dead volume of robot pipette.

Manual washing:

Discard liquid from wells by inverting the plate. Knock the microwell frame with wells downside vigorously on clean adsorbent paper. Pipette 300 µl of diluted wash buffer into each well, wait for 20 seconds. Repeat the whole procedure twice again.

Microplates:

Calculate the number of wells required for the test. Remove unused wells from the frame, replace and store in the provided plastic bag, together with desiccant, seal tightly (2-8°C/35.6-46.4°F).

7.2 Pipetting Scheme

We suggest pipetting calibrators, controls and samples as follows:

For *QUANTITATIVE* interpretation

	1	2	3	4...
A	Cal A	Cal E	P1	
B	Cal A	Cal E	P1	
C	Cal B	Cal F	P2	
D	Cal B	Cal F	P2	
E	Cal C	PC	P3	
F	Cal C	PC	P3	
G	Cal D	NC	...	
H	Cal D	NC	...	

CalA: calibrator A

CalB: calibrator B

CalC: calibrator C

CalD: calibrator D

CalE: calibrator E

CalF: calibrator F

PC: positive control



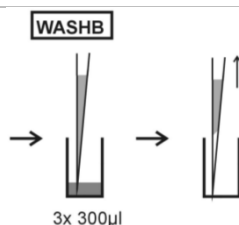
NC: negative control

P1: patient 1


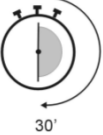
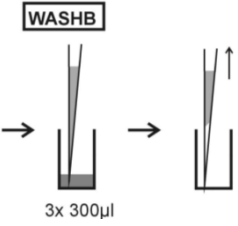


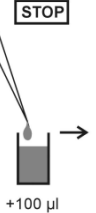

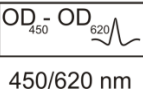
P2: patient 2

P3: patient 3

7.3 Test Steps

Step	Description
1.	Ensure preparations from step 7.1 above have been carried out prior to pipetting.
2.	Use the following steps in accordance with quantitative interpretation results desired:
CONTROLS & SAMPLES	
3.	 <p>Pipette into the designated wells as described in chapter 7.2 above, 100 µl of either: Calibrators (CAL.A to CAL.F) and 100 µl of each of the following:</p> <ul style="list-style-type: none"> Negative control (NC) and Positive control (PC), and Patients diluted serum (P1, P2...)
4.	 <p>Incubate for 30 minutes at 20-32°C/68-89.6°F.</p>
5.	 <p>Wash 3x with 300 µl washing buffer (diluted 1:50).</p>

 autoimmune diagnostic assays	Product Ref.	10302
	Product Desc.	PR3
	Manual Rev. No.	006: 2024-02-13

CONJUGATE		
6.	 +100 µl	Pipette 100 µl conjugate into each well.
7.	 30'	Incubate for 30 minutes at 20-32°C/68-89.6°F.
8.	 3x 300µl	Wash 3x with 300 µl washing buffer (diluted 1:50).
SUBSTRATE		
9.	 +100 µl	Pipette 100 µl TMB substrate into each well.
10.	 30'	Incubate for 30 minutes at 20-32°C/68-89.6°F, protected from intense light.
STOP		
11.	 +100 µl	Pipette 100 µl stop solution into each well, using the same order as pipetting the substrate.
12.	 5'	Incubate 5 minutes minimum.
13.		Agitate plate carefully for 5 sec.
14.	 450/620 nm	Read absorbance at 450 nm (recommended 450/620 nm) within 30 minutes.

8 Quantitative Interpretation

For **quantitative interpretation** establish the standard curve by plotting the **optical density (OD) of each calibrator (y-axis)** with respect to the corresponding concentration values in U/ml (x-axis). For best results we recommend log/lin coordinates and 4-Parameter Fit. From the OD of each sample, read the corresponding antibody concentrations expressed in U/ml.

Normal Range	Equivocal Range	Positive Results
< 12 U/ml	12 - 18 U/ml	>18 U/ml

Example of a standard curve

Do NOT use this example for interpreting patient's result

Calibrators IgG	OD 450/620 nm	CV % (Variation)
0 U/ml	0.040	0.7
3 U/ml	0.175	2.7
10 U/ml	0.350	1.8
30 U/ml	0.680	3.4
100 U/ml	1.220	0.4
300 U/ml	2.130	0.8

Example of calculation

Patient	Replicate (OD)	Mean (OD)	Result (U/ml)
P 01	0.557/0.572	0.565	23.0
P 02	0.998/1.045	1.022	63.8

Samples above the highest calibrator range should be reported as >Max. They should be diluted as appropriate and re-assayed. Samples below calibrator range should be reported as < Min.

For lot specific data, see enclosed quality control leaflet. Medical laboratories might perform an in-house quality control by using own controls and/or internal pooled sera, as foreseen by national regulations.

Each laboratory should establish its own normal range based upon its own techniques, controls, equipment and patient population according to their own established procedures.

In case that the values of the controls do not meet the criteria the test is invalid and has to be repeated.

The following technical issues should be verified: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, photometer, incubation conditions and washing methods.

If the items tested show aberrant values or any kind of deviation or that the validation criteria are not met without explicable cause please contact the manufacturer or the supplier of the test kit.

9 Technical Data

Sample material:	serum
Sample volume:	10 µl of sample diluted 1:101 with 1x sample buffer
Total incubation time:	90 minutes at 20-32°C/68-89.6°F
Calibration range:	0-300 U/ml
Analytical sensitivity:	1.39 U/ml
Storage:	at 2-8°C/35.6-46.4°F use original vials only.
Number of determinations:	96 tests

10 Performance Data

10.1 Normal Range

Sera of healthy donors have been investigated on PR3 and resulted in the following distribution:

Number of Samples	negative	borderline	positive
200	175 (87.5 %)	18 (9 %)	7 (3.5 %)

We also recommend that each laboratory should establish its own normal range.

10.2 Precision

Precision of test results obtained with PR3, REF 10302 were assessed by the determination of the intra- and inter assay precision as well as the lot-to-lot variance by the analysis of multiple samples of different antibody activities.

Sample ID	Intra Assay Precision		Inter Assay Precision		LOT to LOT Precision	
	Mean (U/ml)	CV	Mean (U/ml)	CV	Mean (U/ml)	CV
Sample 1	11.3	10.9%	11.3	13.1%	12.5	0.1%
Sample 2	21.6	7.0%	21.6	8.9%	23.2	8.8%
Sample 3	28.8	10.6%	28.8	12.5%	30.8	16.3%
Sample 4	92.2	8.1%	92.2	8.7%	104.2	16.1%
Sample 5	213.6	10.0%	213.6	10.2%	236.7	18.0%
Sample 6	331.7	5.9%	331.7	6.5%	375.5	17.7%

10.3 Sensitivity and Specificity

Analytical sensitivity

The analytical sensitivity has been assessed by multiple analysis of sample buffer and low positive samples and calculating the limit of detection.

For PR3, REF 10302 a **LoD of 1.39 U/ml** has been determined.

	Product Ref.	10302
	Product Desc.	PR3
	Manual Rev. No.	006: 2024-02-13

10.4 Linearity

Three sera covering the whole test range were diluted serially with a negative serum sample. Measured and expected values of the distinct dilutions were used to calculate a linear regression. According to results of linearity testing a measurable range of 3 - 300 U/ml was determined for PR3.

10.5 Calibration




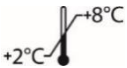

Due the lack of international reference calibration this assay is calibrated in arbitrary units (U/ml).

11 Disposal

Please observe the relevant statutory requirements!

12 Literature

- Falk, RJ Jennette JC (1988).** *Antineutrophil cytoplasmic autoantibodies with specificity for myeloperoxidase in patients with systemic vasculitis and idiopathic necrotizing and crescentic glomerulonephritis.* N Engl J Med 318: 1651-1657.
- Baslund B and Wiik A (1994).** *Anti-neutrophil cytoplasmic autoantibodies (ANCA) and vasculitis.* Clin Rev Allergy; 12: 297-304.
- Bosch X, Guilabert A, and Font J (2006).** *Antineutrophil cytoplasmic antibodies.* Lancet; 368: 404-418.
- Savige J, Gillis D, Benson et al. (1999).** *International consensus statement on testing and reporting of antineutrophil cytoplasmic antibodies (ANCA).* Am J Clin Pathol; 111: 507–13.
- Campanelli D, Detmers PA, Nathan CF and Gabay J E (1990).** *Azurocidin and a homologous serine protease from neutrophils. Differential antimicrobial and proteolytic properties.* J Clin Invest; 85: 904-915.
- Kao RC, Wehner NG, Skubitz KM, Gray BH and Hoidal JR (1988).** *Proteinase 3. A distinct human polymorphonuclear leukocyte proteinase that produces emphysema in hamsters.* J Clin Invest; 82: 1963-1973.
- Rao NV, Wehner NG, Marshall BC, Gray WR, Gray BH and Hoidal JR (1991).** *Characterization of proteinase-3 (PR-3), a neutrophil serine proteinase. Structural and functional properties.* J Biol Chem; 266: 9540-9548.
- Reinhold-Keller E, BeugeN , LatzaU, de Groot K, Rudert H, Nolle B, Heller M and Gross WL (2000).** *An interdisciplinary approach to the care of patients with Wegener's granulomatosis: long-term outcome in 155 patients.* Arthritis Rheum; 43: 1021-1032.
- Aries PM, Csernok E and Gross (2007).** *Antineutrophil cytoplasmic autoantibodies with specificity for proteinase 3.* in Autoantibodies (ed. Shoenfeld Y, Gershwin ME, Meroni PL) (2007) 2nd Edition 119-126
- Lothar Thomas:** Labor und Diagnose. Indikation und Bewertung von Laborbefunden für die medizinische Diagnostik., 8. Auflage, TH Books
- CLSI Guideline GP44-A4:** Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests

IVD	- Diagnosi in vitro - Pour diagnostic in vitro - In Vitro Diagnostikum - Para uso Diagnóstico in vitro	- For in vitro diagnostic use - Para uso diagnóstico in vitro - In Vitro Διαγνωστικό μέσο
REF	- Numero d'ordine - Référence Catalogue - Bestellnummer - Número de catálogo	- Catalogue number - Numéro de catálogo - Αριθμός παραγγελίας
LOT	- Descrizione lotto - Lot - Chargen Bezeichnung - Lote	- Lot - Lote - Χαρακτηρισμός παρτίδας
UDI	- Identificatore univoco del dispositivo - Identifiant unique de l'appareil - eindeutige Produktidentifizierung - Identificador único do dispositivo	- Unique Device Identifier - Identificador único del dispositivo - Μοναδικό αναγνωριστικό συσκευής
CE	- Conformità europea - Déclaration CE de Conformité - Europäische Konformität - Declaração CE de Conformidade	- EC Declaration of Conformity - Declaración CE de Conformidad - Ευρωπαϊκή συμφωνία
	- 96 determinazioni - 96 tests - 96 Bestimmungen - 96 Testes	- 96 tests - 96 pruebas - 96 προσδιορισμοί
	- Rispettare le istruzioni per l'uso - Voir les instructions d'utilisation - Gebrauchsanweisung beachten - Ver as instruções de uso	- See instructions for use - Ver las instrucciones de uso - Λάβετε υπόψη τις οδηγίες χρήσης
	- Da utilizzarsi entro - Utiliser avant le - Verwendbar bis - Utilizar antes de	- Use by - Utilizar antes de - Χρήση μέχρι
	- Conservare a 2-8°C - Conserver à 2-8°C - Lagerung bei 2-8°C - Conservar entre 2-8°C	- Store at 2-8°C (35.6-46.4°F) - Conservar a 2-8°C - Φυλάσσεται στους 2-8°C
	- Prodotto da - Fabriqué par - Hergestellt von - Fabricado por	- Manufactured by - Fabricado por - Κατασκευάζεται από
CON +	- Controllo positivo - Contrôle Positif - Positiv Kontrolle - Controllo positivo	- Positive Control - Control Positivo - Θετικός ορός ελέγχου
CON -	- Controllo negativo - Contrôle Négatif - Negativ Kontrolle - Controllo negativo	- Negative Control - Control Negativo - Αρνητικός ορός ελέγχου
CAL	- Calibratore - Etalon - Kalibrator - Calibrador	- Calibrator - Calibrador - Αντιδραστήριο βαθμονόμησης
CONJ	- Coniugato - Conjugé - Konjugat - Conjugado	- Conjugate - Conjugado - Σύζευγμα
MP	- Micropiastria rivestita - Microplaque sensibilisée - Beschichtete Mikrotiterplatte - Microplaca revestida	- Coated microtiter plate - Microplaca sensibilizada - Επικαλυμμένη μικροπλάκα
WASHB 50x	- Tampone di lavaggio - Tampon de Lavage - Waschpuffer - Solução de lavagem	- Wash buffer - Solución de lavado - Ρυθμιστικό διάλυμα πλύσης
SUB	- Tampone substrato - Substrat - Substratpuffer - Substrato	- Substrate buffer - Tampón sustrato - Ρυθμιστικό διάλυμα υποστρώματος
STOP	- Reagente bloccante - Solution d'Arrêt - Stopreagenz - Solução de paragem	- Stop solution - Solución de parada - Αντιδραστήριο διακοπής αντίδρασης
SB 5x	- Tampone campione - Tampon Echantillons - Probenpuffer - Diluente de amostra	- Sample buffer - Tampón Muestras - Ρυθμιστικό διάλυμα δειγμάτων